STUDIES IN NON-SPECIFIC COMPLEMENT FIXATION*

II. NON-SPECIFIC COMPLEMENT FIXATION BY NORMAL DOG SERUM

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The present investigation was rendered necessary by our researches in distemper and intestinal parasitism of dogs, both of which included complement-fixation tests, in order to ascertain whether apparently normal and healthy dog serum will fix or absorb complement with lipoidal and bacterial antigens as will normal rabbit serum.[1]

Several years ago Rossi[2] observed that a large percentage of the sera of normal dogs yield non-specific complement-fixation with watery extracts of syphilitic liver as antigen, but this observation has not attracted much attention, probably on account of the relative infrequency with which complement-fixation tests are conducted with dog sera.

During the past year we have tested the sera of about 200 dogs with both lipoidal and various bacterial antigens, with the result that a large percentage were found capable of bringing about some degree of non-specific absorption of complement in a manner comparable to that of normal rabbit serum. Tho perfectly fresh and active normal dog serum may give non-specific complement fixation, the tendency is much increased by heating sera at 56 C. for 30 minutes. During the process of heating (inactivation) normal dog serum quickly develops antihemolytic or anticomplementary properties and indeed this condition may occasionally be found in perfectly fresh serum; but the object of this communication is to direct attention to the observation that the sera of normal dogs may show positive or non-specific complement-fixation when this antihemolytic or anticomplementary property is not in evidence.

METHOD OF STUDY

The technic employed was exactly similar to that used by us in conducting the Wassermann syphilis reaction with human serum and

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[1] Kolmer and Casselman: Jour. Med. Research, 1913, 28, p. 369. Kolmer and Trist: Jour. Infect. Dis., 1916, 18, p. 20.

[2] Ztschr. f. Immunitätsf., R., 1909, 1, p. 429.

in a similar study of non-specific complement fixation with normal rabbit serum.

Sera.—The animals were kept on an ordinary mixed diet and bled from the external jugular vein. The sera were tested while in a fresh, active condition, and again after heating at 56 C. for half an hour. In the majority of tests the sera were used in 0.1 c.c. amounts, but for purposes of a quantitative study a number were tested in amounts of 0.05, 0.1, 0.2, and 0.4 c.c.

Many of the dogs examined showed some evidences of distemper, and it is not improbable that a proportion of the older animals had distemper prior to admission to the kennels, but in complement-fixation the sera of young and apparently healthy dogs showed in a similar manner a tendency to absorb complement with various lipoidal and bacterial antigens, so that in this study we have included all animals under the heading of "normal," altho distemper or some other infection prior to these tests may have had an influence upon the results with our bacterial antigens. This subject will be discussed in a later communication; here are recorded the results of complement-fixation tests with the sera of dogs of various ages ordinarily met with in the kennels.

Antigens.—Both lipoidal extracts and bacterial emulsions were employed. The lipoidal extracts were the three used in our routine Wassermann reactions: an alcoholic extract of human heart re-enforced with cholesterin; an alcoholic extract of syphilitic liver; and an extract of acetone insoluble lipoids. The doses of these, determined at intervals by titration with luetic sera, equalled twice their antigenic units. These doses were at least from 6 to 10 times less than the anticomplementary units.

Three bacterial antigens were employed; namely, polyvalent emulsions of washed 24-hour agar cultures of staphylococci, human colon bacilli, and typhoid bacilli. The staphylococcus antigen was prepared from 5 strains of Staphylococcus aureus from abscesses and 6 strains of Staphylococcus albus from acne vulgaris—all from human infections; the colon antigen was prepared from 6 strains of B. coli communis from human feces and the typhoid antigen from 2 strains of B. typhosus. The emulsions were shaken mechanically for several hours, filtered through paper, heated at 60 C. for an hour, and preserved with 0.25% phenol. Each of these antigens was titrated before the complement-fixation tests, and used in amounts equalling one-quarter of their anticomplementary doses.

The doses of antigen employed are important as the percentage of positive reactions may be altered by using amounts larger or smaller than those mentioned.

Technic.—The antisheep hemolytic system was used throughout. Complement was furnished by the pooled sera of 2 or more guinea-pigs and used in dose of 1 c.c. of a 1:20 dilution (0.05 c.c. undiluted serum). The hemolysin was titrated in increasing doses against this unit of complement serum and 1 c.c. of a 2.5% suspension of washed sheep erythrocytes, prepared for each day's work and used in the antigen titrations and main tests in an amount equal to double the hemolytic unit. Less than this amount of hemolysin increases the percentage of positive reactions and the anticomplementary tendencies of the serum alone.

Serum, antigen, complement, and sufficient salt solution were incubated for an hour at 37 C.; then 2 units of hemolysin and 1 c.c. of the erythrocyte suspension were added; after mixing, the tubes were re-incubated for an hour or an hour and a half, depending upon the hemolysis of the controls, and then

placed in the refrigerator over night, the readings being made the following morning. As customary, a control on every serum, antigen, and complement, and hemolytic controls, were included.

SUMMARY OF RESULTS

Dog serum in both an active or fresh condition and after heating or inactivation is capable of absorbing complement, in a relatively large percentage of instances, with various lipoidal and bacterial antigens.

The animals tested were those ordinarily met with in the kennels, and while a number of these had or probably had had distemper, most of them, tested within a short time after admission, showed no evidences of this infection. In this series no differences in complement fixation with the antigens employed were noted between the sera of normal dogs and the sera of those showing the symptoms of distemper, and the results have been ascribed to a process of non-specific complement fixation or absorption.

TABLE 1

THE PERCENTAGES OF POSITIVE REACTIONS WITH INCREASING DOSES OF ACTIVE DOG SERUM AND VARIOUS LIPOIDAL AND BACTERIAL ANTIGENS

Antigen	Percentage of Positive Reactions			
	0.05 c.c. Serum	0.1 c.c. Serum	0.2 c.c. Serum	0.4 c.c. Serum
Cholesterinized alcoholic extract of human heart	29	16	0	0
Alcoholic extract of syphilitic liver	25	8	0	0
Acetone insoluble lipoids	12.5	4	0	0
Staphylococci	96	54	75	25
B. coli (human)	90	66	50	0
B. typhosus	91	70	41	0

As shown in Tables 1 and 2, the highest number of positive reactions was observed with the bacterial antigens. With heated sera from normal dogs it was the exception rather than the rule to observe a negative reaction with these antigens and this has a very important bearing upon the question of complement-fixation tests with dog serum for diagnostic purposes, as in the complement-fixation test for distemper.

Among the lipoidal extracts, the highest percentage of positive reactions occurred with alcoholic extract of human heart re-enforced with cholesterin; next in the order of yielding positive reactions came an alcoholic extract of syphilitic liver, while an extract of

acetone insoluble lipoids yielded the lowest percentage of positive reactions. These relations held with both active and inactivated sera, and the results are similar to those observed with normal rabbit serum. It would appear that the presence of cholesterin in an extract increases the percentage of positive reactions, as it undoubtedly enhances the antigenic sensitiveness of any tissue extract for the syphilis reagin in the Wassermann reaction.

As shown in Table 1, fresh, active dog serum in dose of 0.05 c.c. yielded the highest percentage of positive reactions with both lipoidal and bacterial antigens; while with doses of 0.2 and 0.4 c.c. of serum no reactions occurred with the lipoidal extracts, and with the bacterial antigens the tendency for complement fixation was greatly reduced. It is probable that native hemolytic complement and antisheep hemolysin present in the dog sera were partly responsible for these results and obscured lesser degrees of complement absorption.

TABLE 2

THE PERCENTAGES OF POSITIVE REACTIONS WITH INCREASING DOSES OF HEATED (INACTIVATED) DOG SERUM AND VARIOUS LIPOIDAL AND BACTERIAL ANTIGENS

Antigen	Percentages of Positive Reactions			
	0.05 c.c. Serum	0.1 c.c. Serum	0.2 c.c. Serum	0.4 c.c. Serum
Cholesterinized alcoholic extract of human heart	39	63	60	50
Alcoholic extract of syphilitic liver	25	50	48	50
Acetone insoluble lipoids	26	21	36	30
Staphylococci	90	96	100	70
B. coli (human)	73	80	88	50
B. typhosus	40	76	87	70

Heating dog serum at 55 C. for 30 minutes greatly increases the tendency toward non-specific complement fixation, not only with lipoidal, but also with the bacterial, antigens. This is at once apparent in comparing Tables 1 and 2. The tendency toward absorption or fixation of complement and the percentage of positive reactions increase with increasing doses of serum, except when 0.4 c.c. serum is used, when the tendency somewhat diminishes as a result to some degree of a masking of complement fixation by the presence of natural or normal antisheep hemolysins in dog serum.

The degree or amount of complement absorption with lipoidal extracts is usually moderate or slight, as measured by the method

employed. This is shown in Table 3, which summarizes the percentages of positive reactions showing 50%, or less, inhibition of hemolysis with the various antigens. With the cholesterinized alcoholic extract of heart and alcoholic extract of syphilitic liver, more complement was absorbed than with the extract of acetone insoluble lipoids; the bacterial antigens showed a greater degree of complement absorption inasmuch as the reactions were more frequently ++++ and +++ than ++ or less.

TABLE 3

THE PERCENTAGES OF POSITIVE REACTIONS OF 50% (++), OR LESS, INHIBITION OF HEMOLYSIS WITH 0.1 C.C. OF ACTIVE AND INACTIVATED DOG SERA AND VARIOUS ANTIGENS

Antigens	Serum (0.1 c.c.)	
	Active	Inactivated
Cholesterinized alcoholic extract of human heart	70	43
Alcoholic extract of syphilitic liver	77	68
Acetone insoluble lipoids	100	94
Staphylococci	45	32
B. coli (human)	41	42
B. typhosus	28	50

DISCUSSION

Without discussing at this time the mechanism of non-specific complement fixation with dog serum, we would point out the tendency which dog serum shows in this connection, and urge great caution in the conduct and interpretation of complement-fixation tests with it.

While dog serum is not infrequently antihemolytic or anticomplementary after inactivation, even when fresh and shortly after bleeding, we have not included any of these sera in this work, so that the positive reactions are to be interpreted as evidences of complement fixation or absorption between the antigens and some substance in the serum. As with rabbit serum, dog serum develops this property of fixing complement in a non-specific manner as the result of heating the serum at 56 C. for one-half hour, altho fresh, active serum may also show the phenomenon, as indicated in the tables.

When it is desired to conduct complement-fixation tests with dog serum for specific amboceptors, it would appear advisable to use the serum in a perfectly fresh and active condition in dose of 0.1 to 0.2 c.c.; or, after heating the serum at 62 C. instead of 55 C. for half an hour, since this, as will be pointed out later, removes, or greatly diminishes, the tendency toward non-specific fixation of complement.